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VERSITA

Ornithodoros quilinensis sp. nov. (Acari, Argasidae), a new tick species from the Chacoan region in Argentina

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Abstract

Ornithodoros quilinensis sp. nov. (Acari: Argasidae) is described from larvae collected on the small rodents *Graomys centralis* (Cricetidae: Sigmodontinae) in Argentina. The diagnostic characters for this new species are a combination of small size (520–540 µm), a dorsal plate oval in shape with a length of approximately 200 µm, 14 pairs of dorsal setae, hypostome short and narrower at the base (length from Ph₁ to apex 133 µm (120–141)) with dental formula 2/2 and apex blunt, and the capsule of the Haller's organ irregular in shape and without reticulations. The analysis of the 16S rDNA sequences available for the genus *Ornithodoros* indicate that, phylogenetically, *O. quilinensis* represents an independent lineage only related to a Bolivian tick species of the genus *Ornithodoros* yet not formally described.

Keywords

Ornithodoros quilinensis, Argasidae, Chacoan region, Argentina

Introduction

Currently there are 899 species of ticks described around the world, 195 species are included in the family Argasidae (soft ticks), 703 species belong to the family Ixodidae (hard ticks), and the only member of the family Nuttalliellidae is the African species *Nuttalliella namaqua* Bedford, 1931 (Guglielmone *et al.* 2010, Nava *et al.* 2010, Apanaskevich *et al.* 2011, Dantas-Torres *et al.* 2012). Specifically for the Neotropical region, the specific richness of ticks is represented by 116 species of hard ticks (Guglielmone *et al.* 2003, Labruna *et al.* 2005, Barros-Battesti *et al.* 2007, Nava *et al.* 2009) and 83 of soft ticks (Guglielmone *et al.* 2003, Labruna and Venzal 2009, Nava *et al.* 2010, Dantas-Torres *et al.* 2012).

As a result of the increment of the number of studies focused on soft ticks in the last 10 years in South America, several new species of the family Argasidae were described in this continent using both morphological and molecular characters. In this way, the recent description of new species belonging to different genera [*Argas keiransi* Estrada-Peña, Venzal et González-Acuña, 2003; *Antricola guglielmonei*

Estrada-Peña, Barros-Battesti et Venzal, 2004; *Antricola delacruzi* Estrada-Peña, Barros-Battesti et Venzal, 2004; *Antricola inexpectata* Estrada-Peña, Barros-Battesti et Venzal, 2004; *Ornithodoros rondoniensis* (Labruna, Terassini, Camargo, Brandão, Ribeiro et Estrada-Peña, 2008); *Ornithodoros rioplatensis* Venzal, Estrada-Peña et Mangold, 2008; *Ornithodoros fonsecai* (Labruna et Venzal, 2009); *Nothoaspis amazoniensis* Nava, Venzal et Labruna, 2010; *Ornithodoros cavernicolous* Dantas-Torres, Venzal et Labruna, 2012] has put in evidence that the diversity of argasid ticks in the Neotropics is probably underestimated.

To date, only 4 species of the genus *Ornithodoros* Koch, 1844 have been recorded in Argentina, namely *Ornithodoros mimon* Kohls, Clifford et Jones, 1969; *Ornithodoros rostratus* Aragão, 1911; *Ornithodoros hasei* (Schulze, 1935) and *O. rioplatensis* (Guglielmone and Nava 2005, Nava *et al.* 2007, Venzal *et al.* 2008). Dios and Knopoff (1930) cited *Ornithodoros turicata* (Dugès, 1876) for Argentina, but Aragão (1935) determined these ticks as *O. rostratus*. In the same way, Boero (1957) and Capriles and Gaud (1977) mentioned the presence of *Ornithodoros talaje* (Guérin-Méneville, 1849) and *Ornithodoros puertoricensis* Fox, 1947 in Argentina, respec-

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tively, but Venzal *et al.* (2008) suggested that these records correspond to *O. rioplatensis*. The aim of this work is to describe a new tick species of *Ornithodoros* from specimens collected on small rodents in a locality belonging to the Chacoan region of Argentina.

Materials and methods

Ninety-six larvae of ticks belonging to the genus *Ornithodoros* were collected on specimens of the small rodent *Graomys centralis* (Thomas, 1902) (Rodentia: Cricetidae) in Quilino (30°

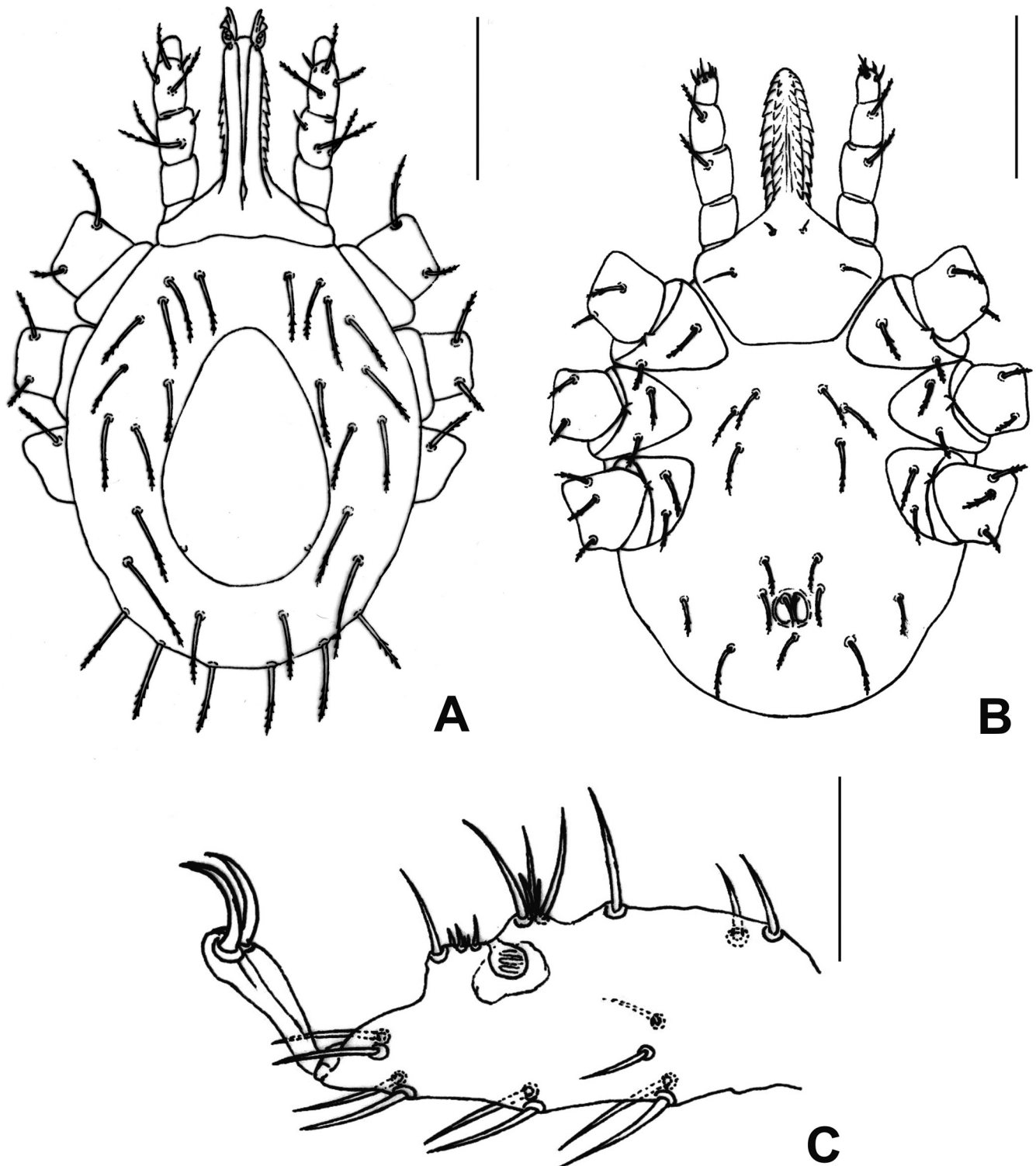


Fig. 1. *Ornithodoros quilinensis* sp. nov. larva. **A** – dorsal. **B** – ventral. **C** – tarsus I. Scale bars = 150 μ m (A-B), 50 μ m (C)

12°S, 64°32'W), Córdoba Province, Argentina, on April 20, 2010. Additionally, 4 larvae morphologically identical were found on *G. centralis* in the same locality at March 22, 2011. This locality lies in a dry area of the Western Chaqueño District located in the Chaco phytogeographic province according to the phytogeographic divisions described by Cabrera (1994).

Ticks were cleaned with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1. Twenty-seven slightly or not engorged larvae were mounted

in Hoyer's medium to create semi-permanent slides for light microscopy. Ten larvae were measured using a Nikon Eclipse E200 optical microscope. All measurements are given in micrometres (μm), with the mean followed by the standard deviation and range in parentheses. In the descriptions, larval chaetotaxic terminology and measures followed Sonenshine *et al.* (1962) and Kohls *et al.* (1965), with the modifications proposed by Venzal *et al.* (2008) and Labruna *et al.* (2011).

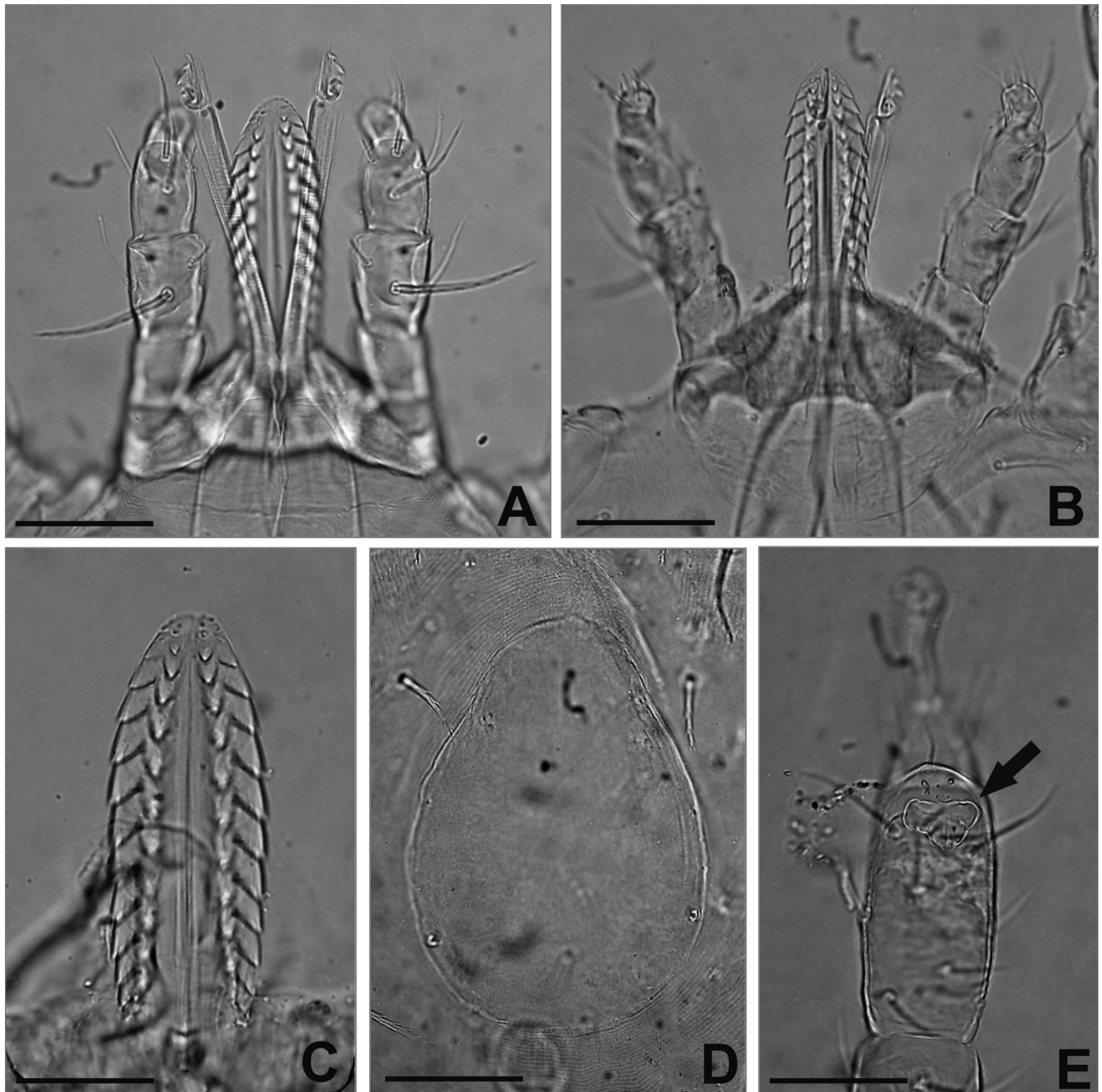


Fig. 2. *Ornithodoros quilinensis* sp. nov. larva. **A** – capitulum dorsal. **B** – capitulum ventral. **C** – hypostome. **D** – dorsal plate. **E** – capsule of Haller's organ (dorsal view). Scale bars = 75 μm (A-D), 100 μm (B), 50 μm (C-E).

DNA was extracted from 2 larvae (one collected in 2010 and other in 2011) and processed using a polymerase chain reaction (PCR). A 460-bp fragment of the mitochondrial 16S rDNA gene was amplified with the primers designed by Mangold *et al.* (1998). Each of the sequences was aligned with each other and with the corresponding sequences of the *Ornithodoros* species available in GenBank, using the BioEdit Sequence Alignment Editor (Hall 1999) with the CLUSTAL W program (Thompson *et al.* 1994). Phylogenetic relationships were assessed in terms of neighbour-joining distances (NJ) and maximum parsimony (MP) methods using MEGA version 4.0 (Tamura *et al.* 2007). The NJ tree was generated from the Tamura-Nei model and gaps were excluded in the pairwise comparison. MP analysis was performed using the heuristic search procedure, with the close neighbour interchange (CNI) set at search level 3 and the random addition of taxa (10 replicates), and gaps were excluded from the analysis. Support for the NJ and MP topology was tested by bootstrapping over 1,000 replications. The sequences of *Argas neghmei* Kohls et Hoogstraal, 1961 and *A. keiransi* were used as outgroups.

At the present, there is not consensus among tick researchers about classification of soft ticks at the genus level (Estrada Peña *et al.* 2010). Therefore, we follow the classification presented by Guglielmone *et al.* (2010) only to locate the species analyzed in a classificatory scheme, but not in order to support this classification instead of those proposed by other authors (see Estrada Peña *et al.* 2010).

Results

Ixodida Leach, 1815

Argasidae Canestrini, 1890

Ornithodoros Koch, 1844

***Ornithodoros quilinensis* Venzal, Nava et Mangold (Figs 1–2)**

Larva

Body: Idiosoma oval; length with capitulum 525 ± 25 (503–579), length without capitulum 358 ± 28 (332–427), width 325 ± 42 (247–380).

Dorsum: Dorsal plate oval, length 200 ± 17 (172–221), width 152 ± 7 (141–168). Dorsal surface provided with 14 pairs of setae, 7 anterolateral, 3 central and 4 posterolateral. Anterolateral setae (Al): Al₁ length 60 ± 3 (55–65), Al₂ length 62 ± 2 (60–67), Al₃ length 60 ± 5 (53–67), Al₄ length 61 ± 5 (53–67), Al₅ length 64 ± 5 (57–72), Al₆ length 66 ± 4 (57–74), Al₇ length 70 ± 3 (67–74). Central setae (C): C₁ length 51 ± 3 (48–55), C₂ length 73 ± 4 (67–79), C₃ length 71 ± 4 (62–77). Posterolateral setae (Pl): Pl₁ length 79 ± 4 (72–84), Pl₂ length 79 ± 6 (69–89), Pl₃ length 77 ± 4 (67–84), Pl₄ length 77 ± 5 (67–84).

Venter: Ventral surface provided with 7 pairs of setae plus 1 pair on anal valves, 1 posteromedian seta present. Three

pairs of sternal setae (St): St₁ length 46 ± 2 (41–50), St₂ length 45 ± 2 (41–48), St₃ length 44 ± 3 (38–50); one pair of postcoxal setae (Pc) length 38 ± 4 (31–43); three pairs of circumanal setae (Ca): Ca₁ length 40 ± 2 (36–43), Ca₂ length 40 ± 3 (36–45), Ca₃ length 50 ± 5 (38–57); posteromedian setae (PM) length 42 ± 5 (36–50).

Capitulum: Basis capituli pentagonal, posterior margin straight, length from posterior margin of basis capituli to posthypostomal setae: Ph₁ 91 ± 3 (86–96), length from posterior margin of basis capituli to insertion of hypostome 104 ± 4 (98–113), length from posterior margin of basis capituli to apex of hypostome 222 ± 6 (209–230), width 151 ± 5 (144–156). Two pairs of posthypostomal setae; Ph₁ length 14 ± 2 (12–17), Ph₂ length 31 ± 2 (29–36), distance between Ph₁ setae 16 ± 2 (14–19), distance between Ph₂ setae 74 ± 5 (65–86). Palpi total length 143 ± 6 (132–151), segmental length/width from I–IV: (I) 37 ± 1 (36–41)/ 34 ± 2 (31–38), (II) 43 ± 3 (38–48)/ 35 ± 1 (33–38), (III) 43 ± 2 (43–48)/ 31 ± 1 (29–33), (IV) 24 ± 3 (19–29)/ 20 ± 1 (19–21). Setae number on palpal articles I–IV: (I) 0, (II) 4, (III) 5, (IV) 9.

Hypostome: length from Ph₁ to apex 133 ± 7 (120–141), length from insertion of hypostome in basis capituli to apex 118 ± 4 (110–122), width in medial basis portion of hypostome 43 ± 2 (41–48), width in basis portion of hypostome 36 ± 3 (33–41); hypostome narrower at the base, and apex blunt. Dentition formula 2/2 throughout entire length, file 1 with 13 to 15 denticles, file 2 with 11 to 14 denticles, some specimens with 1 accessory file with 1 denticle; corona in apex with 2 very small denticles.

Legs: Tarsus I length 129 ± 2 (127–134), tarsus I width 53 ± 2 (48–57). Setal formula of tarsus I: 1 pair apical (A), 1 distomedian (DM), 5 paracapsular (PC), 1 posteromedian (PM), 1 pair basal (B), 1 pair apicoventral (AV), 1 pair midventral (MV), 1 pair basiventral (BV), and 1 pair posterolateral (PL). Capsule of Haller's organ: irregular in shape, without reticulations.

Taxonomic summary

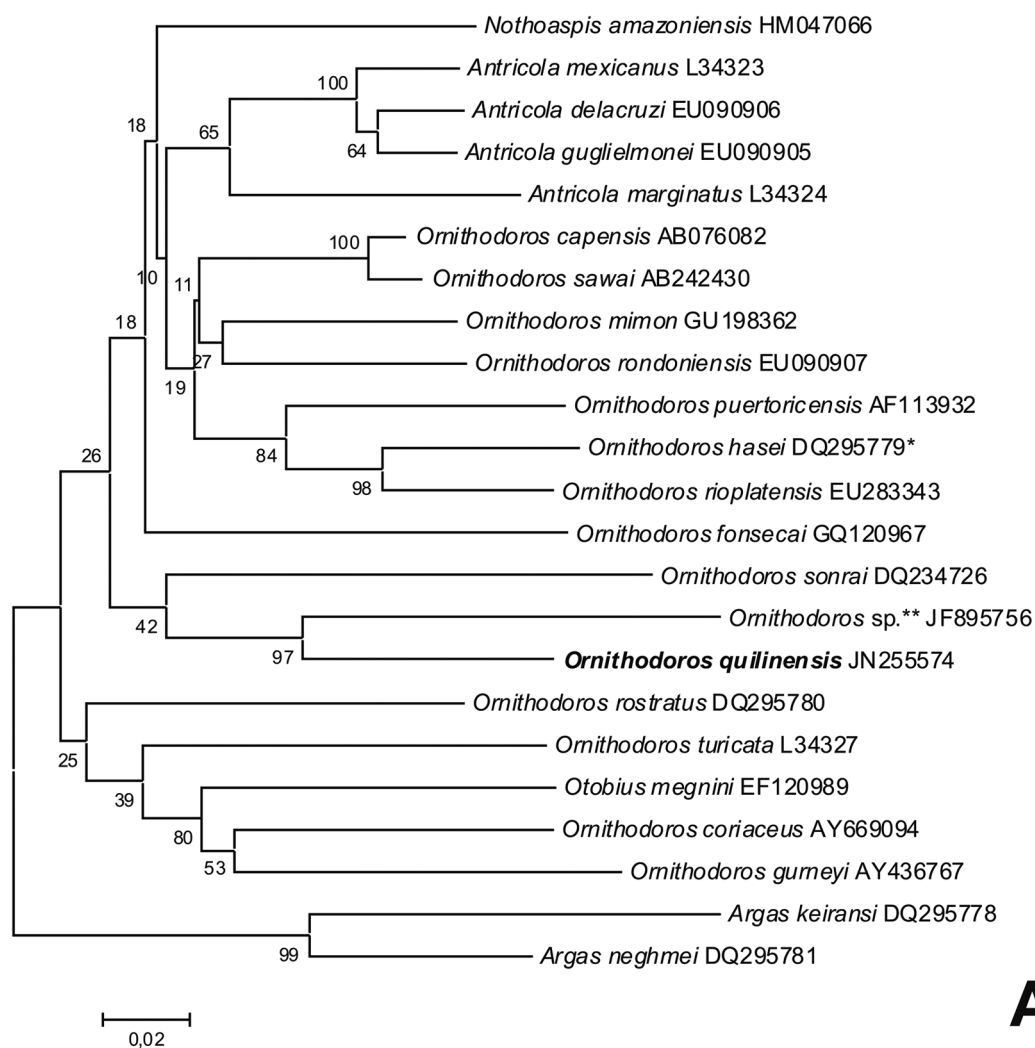
Type host: *Graomys centralis* (Thomas, 1902).

Type locality: Quilino (30°12'S, 64°32'W), Córdoba Province, Argentina.

Type specimens: Holotype larva, allotype larva, 3 paratype larvae mounted in same slide, 5 paratype larvae mounted in other slide and 10 paratype larvae in 70% ethanol, collected on April 20, 2010 by A.J. Mangold, S. Nava, J.M. Venzal and M. Mastropaolo on *G. centralis*, deposited in the U.S National Tick Collection, Georgia Southern University, Statesboro (RML 124999); 5 paratype larvae mounted in slide and 5 in 70% ethanol, same data as for holotype, deposited in the tick collection of INTA Rafaela, Santa Fe, Argentina (INTA 2179); 8 paratype larvae mounted in three slides and 5 in 70% ethanol, same data as for holotype, deposited in the tick collection of the Departamento de Parasitología Veterinaria, Facultad de Veterinaria, Universidad de la República, Monte-

Table I. Comparison of larval morphological characters between *O. quilinensis* sp. nov. and other similar Neotropical *Ornithodoros* species (1)

	<i>Ornithodoros quilinensis</i>	<i>Ornithodoros mimon</i>	<i>Ornithodoros echimys</i>	<i>Ornithodoros casebeeri</i>	<i>Ornithodoros chironectes</i>	<i>Ornithodoros tuttlei</i>	<i>Ornithodoros talaje</i> group
Dorsal plate	oval	pyriform	pyriform	pyriform	pyriform	pyriform	pyriform
Dorsal plate: length	172–221	160–226	196–212	240–288	209–240	275–332	235–300
Dorsal setae: total pairs	14	13–15	14	17–18	16–17	15	17–20
Hypostome: length	120–141	200–220	204–220	232–235	179–200	255–291	200–280
Apex of hypostome	blunt	blunt	rounded	rounded	rounded	rounded	pointed
Dental formula in anterior portion of hypostome	2/2	4/4	4/4	3/3	3/3	5/5	3/3
References	This study	Kohls <i>et al.</i> 1969, Barros-Battesti <i>et al.</i> 2011	Kohls <i>et al.</i> 1969	Jones and Clifford, 1972	Jones and Clifford, 1972	Jones and Clifford, 1972	Venzal <i>et al.</i> 2008

(1) Measurements are in μm .**A**

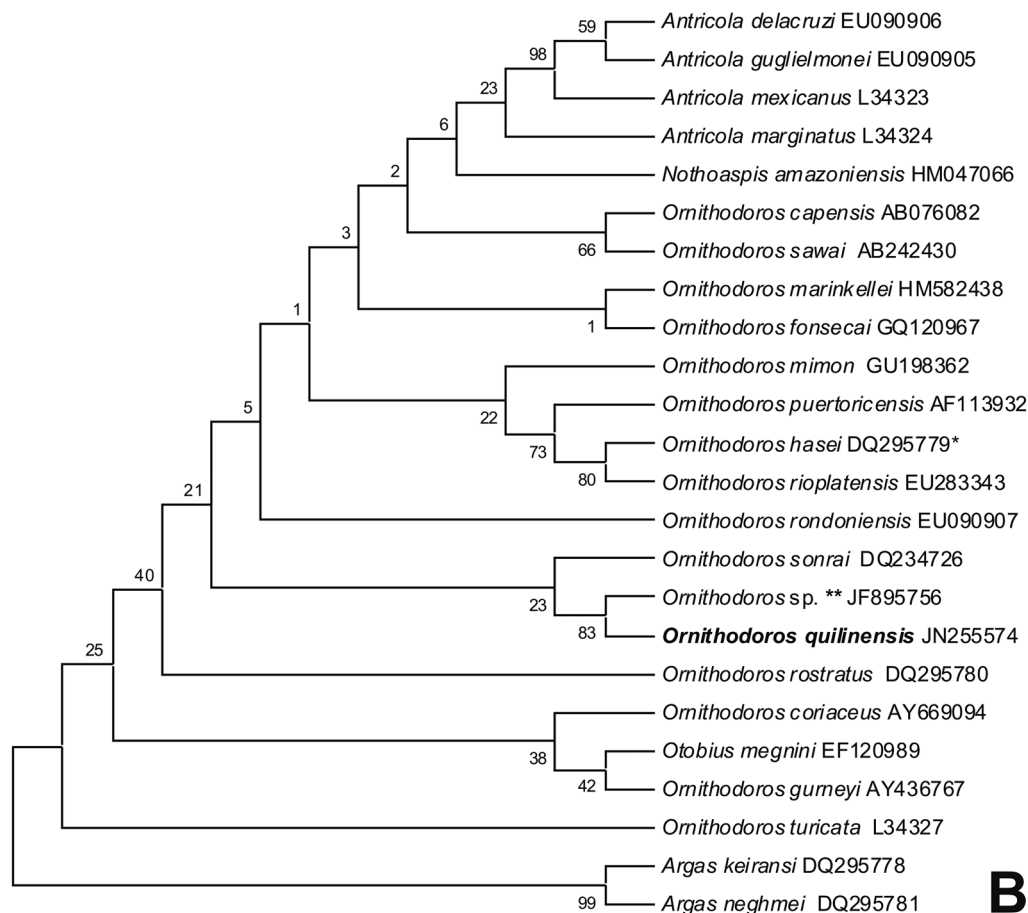


Fig. 3. Neighbour-joining (A) and maximum parsimony (B) trees based on 16S rDNA partial sequences. Numbers represent bootstrap support generated from 1,000 replications. Numbers in brackets are GenBank accession numbers. *The specimen used to obtain this sequence was erroneously classified as *Ornithodoros mimon* when the sequence was submitted to GenBank (J.M. Venzal, pers. comm.). ** Sequence obtained from a tick specimen collected in Bolivia and determined by Parola *et al.* (2011) as *Ornithodoros* sp. According to these authors, this Bolivian tick is probably a new species.

video, Uruguay (DPVURU 769, 770, 771); 2 paratype larvae mounted in slide and 5 in 70% ethanol, same data as for holotype, deposited in the tick collection in the Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil (CNC-1917); 2 paratype larvae mounted in slide and 5 in 70% ethanol, same data as for holotype, deposited in the Instituto Butantan, São Paulo, Brazil (IBSP-10.720). Etymology: The specific epithet “*quilinensis*” refers to the locality (Quilino) where the type specimens were collected.

Species relationship

The diagnostic characters unique for the larva of *O. quilinensis* are its small size (520–540 µm), a dorsal plate oval in shape with a length of approximately 200 µm, 14 pairs of dorsal setae, hypostome short and narrower at the base (length from Ph_1 to apex 133 ± 7 (120–141)) with dental formula 2/2 and apex blunt, and the capsule of the Haller’s organ irregular in shape and without reticulations. The combination of these

morphological characters allows differentiating the larva of *O. quilinensis* from the remaining larvae of the genus *Ornithodoros* that were described in the western hemisphere.

A comparison of larval morphological characters of *O. quilinensis* with other similar Neotropical *Ornithodoros* species is shown in Table I.

The larva of *O. quilinensis* share with *O. mimon* the small size of unengorged specimens, the number of dorsal setae (14 pairs) and the hypostome with apex blunt. However, both species can be easily separated because in *O. mimon* the dental formula in the apex of the hypostome is 4/4 (2/2 in *O. quilinensis*), and the dorsal plate is pyriform in shape (oval in *O. quilinensis*).

Ornithodoros quilinensis is also morphologically related to a group of Neotropical *Ornithodoros* species which are associated to small rodents and marsupials. They share a hypostome with apex blunt, the dorsal plate oval in shape, and the number of dorsal setae. Larvae of *Ornithodoros echimys* Kohls, Clifford et Jones, 1969 and *O. quilinensis* have a dor-

sal plate similar in shape and size, and 14 pairs of dorsal setae, but *O. echimys* has a longer hypostome (200–220 µm) without denticles in its base, and a dental formula 4/4 in anterior portion of hypostome. Most of the records of *O. echimys* correspond to larvae collected on *Echimys semivillosus* (I. Geoffroy, 1838) (Rodentia: Echimyidae) in Venezuela, with the only exception of a record on *Marmosa* sp. (Didelphimorphia: Didelphidae) in Bolivia (Kohls *et al.* 1969; Jones *et al.* 1972). Other species morphologically related to *O. quilinensis* is *Ornithodoros casebeeri* Jones et Clifford, 1972, but they are easily distinguished because *O. casebeeri* has a dental formula 3/3 and anterior projections on the base of hypostome. Larvae of this tick were found on *Ototylomys* sp. (Rodentia: Cricetidae) in Costa Rica and on *Peromyscus stirtoni* Dickey, 1928 (Rodentia: Cricetidae) in Nicaragua (Jones and Clifford 1972). *Ornithodoros chironectes* Jones et Clifford, 1972 is also similar to *O. quilinensis*, but *O. chironectes* has 16–17 pairs of dorsal setae, a dorsal plate pyriform in shape, and dental formula is 3/3 in anterior portion of hypostome. All records of larvae of *O. chironectes* were made on *Chironectes minimus* (Zimmermann, 1780) (Didelphimorphia: Didelphidae) and *Sigmodon hispidus* Say et Ord, 1825 (Rodentia: Cricetidae) in Nicaragua (Jones and Clifford 1972). The other species of this group, *Ornithodoros tuttlei* Jones et Clifford, 1972, is differentiated from *O. quilinensis* by the dental formula 5/5 (*O. quilinensis* has 2/2). The findings of *O. tuttlei* correspond to larvae collected on *Cuniculus paca* (Linnaeus, 1766) (Rodentia: Cuniculidae) and *Tapirus terrestris* (Linnaeus, 1758) (Perissodactyla: Tapiridae) in Venezuela (Jones and Clifford 1972).

Finally, *O. quilinensis* should be compared with the species belonging to the *Ornithodoros talaje* species group, which is formed by *Ornithodoros talaje* (Guérin-Méneville, 1849), *Ornithodoros puertoricensis* Fox, 1947 and *O. rioplatensis* (Venzal *et al.* 2008). Larvae of these 3 species can be distinguished from *O. quilinensis* larvae by the presence of a dorsal plate pyriform, hypostome pointed and dorsal setae more numerous (17–20 pairs in *O. talaje* species group and 14 pairs in *O. quilinensis*).

Molecular analysis

The two sequences of the larvae determined as *O. quilinensis* (GenBank accession numbers JN255574 and JN255575) only differed in one (position 256) of the 449 nucleotides.

The topologies of NJ and MP trees are showed in Figure 3. In both phylogenetic reconstructions, *O. quilinensis* only was closely related with a tick from Bolivia determined by Parola *et al.* (2011) as *Ornithodoros* sp. According to these authors, this Bolivian tick is probably a new species. Unfortunately, there are not available sequences of *O. echimys*, *O. casebeeri* and *O. chironectes*, which share with *O. quilinensis* a similar morphology and the same type of host (small terrestrial mammals). From a phylogenetic perspective, *O. quilinensis* appear to be an independent lineage, only related to a tick species not formally described. However, these results on the phyloge-

netic position of *O. quilinensis* should be considered carefully because the aforementioned lack of available sequences of argasid species and the use of only one gene.

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